

## Motivation & Objective

The use of enzymes as catalyst has increased significantly in recent decades.

Applying pressure can further improve the potential of the enzymes:

- Higher enzyme stability, activity<sup>[1]</sup>, selectivity<sup>[2]</sup>.
- Gas consuming reactions can profit from higher gas solubility.<sup>[3]</sup>

### Utilization of pressure effects through the development of:

- ➔ Low-shear high-pressure reactors.
- ➔ Online and *in situ* analytical methods suitable for high pressure.
- ➔ Characterization of the enzyme activity under pressure within the reactor system by the integrated measurement technology.

## Enzyme Immobilization

- Covalent binding of the enzyme to bioavailable polyamide 12 (PA 12).
- Adaptation of a method for immobilization of tyrosine at PA 12 carrier structures.
- Production through additive manufacturing.
- Use of periodic open cell structures (POCS)<sup>[4]</sup>
- Variation of the POCS geometry:
  - Polyhedra
  - Star Tetrahedron
  - Gyroids.

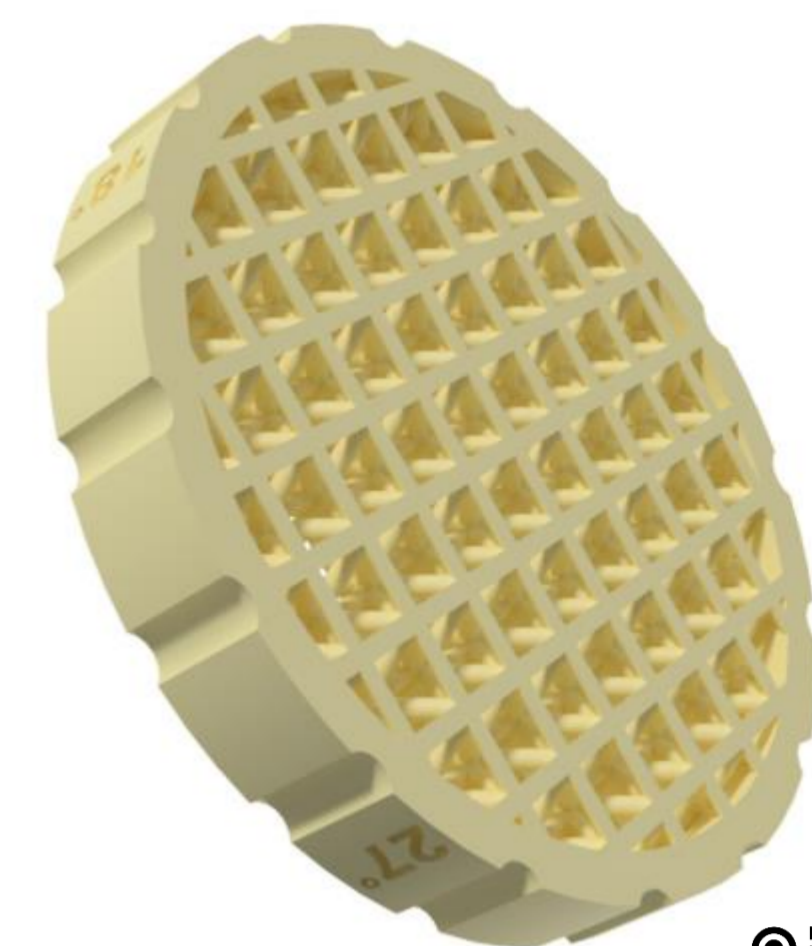


Fig. 3: CAD model of the carrier structure.

## Laboratory Prototype

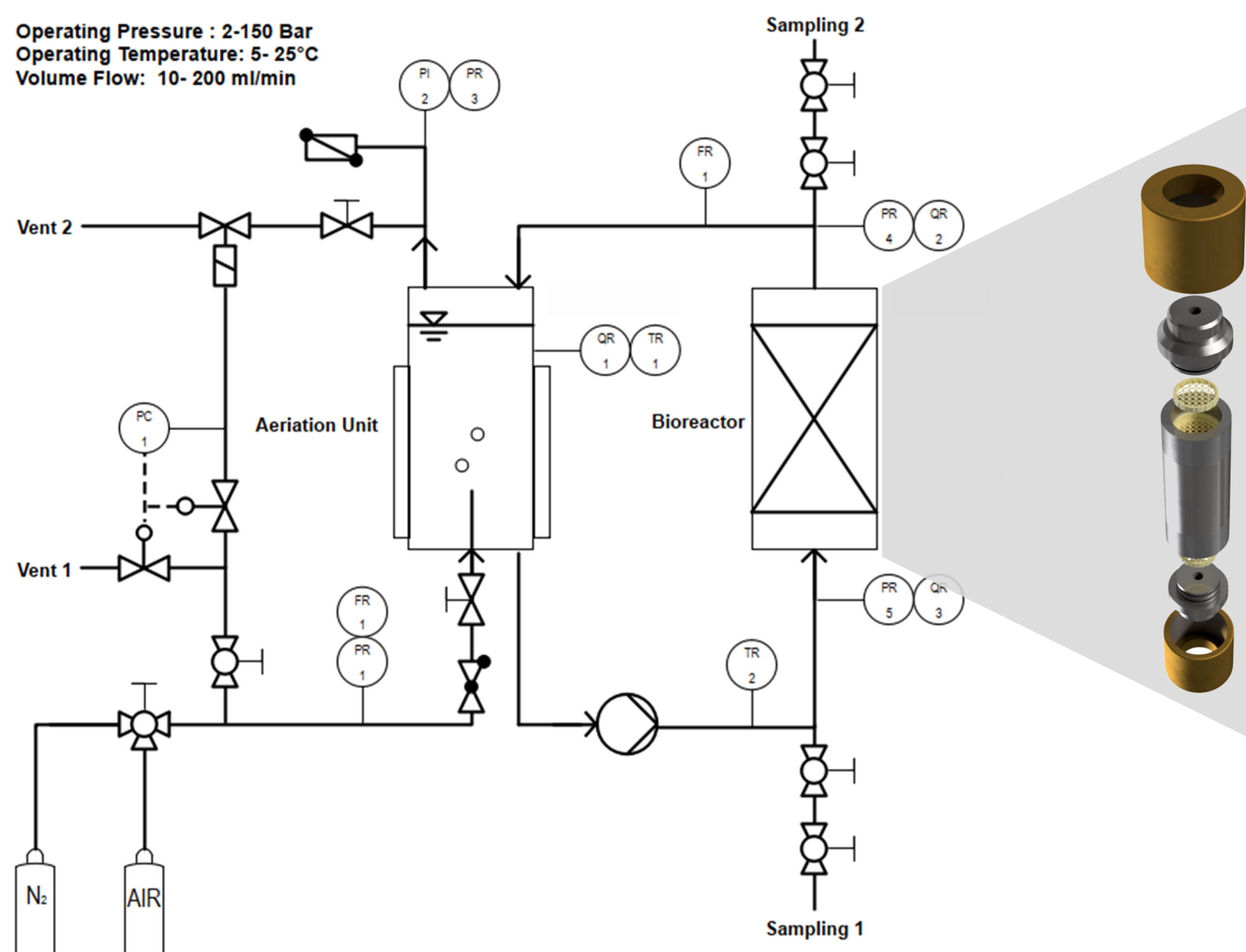


Fig. 1: Flow diagram of the laboratory prototype.

- Reactor setup with spatial separation of aeration and enzymes to minimize shear stress.
- Online and *in situ* monitoring of the oxygen Monitoring enzyme activity via oxygen consumption in the substrate.
- Model reaction is the oxidation of L-tyrosine to L-DOPA by tyrosinase (EC 1.14.18.1).

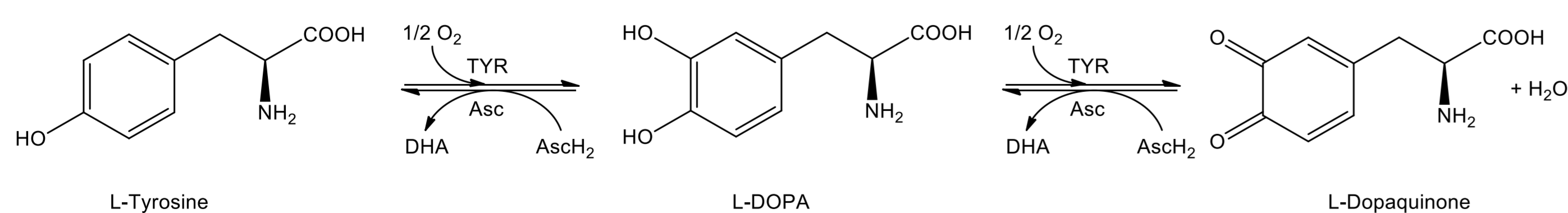


Fig. 2: Reaction equation of the model reaction.

- Tyrosinase is inserted into the bioreactor in immobilized state.

## Results

- In the first project phase a reactor design was developed and tested:
  - Allows a bubble- and therefore foam-free aeration.
  - Allows high turnover without oxygen limitation.
  - Leads to no enzyme damage through shear stress.
- Oxygen depletion can be observed between the sensors at the inlet and outlet of the reactor (Fig. 4)
- The measured consumption is also reflected in the course of the L-tyrosine turnover.

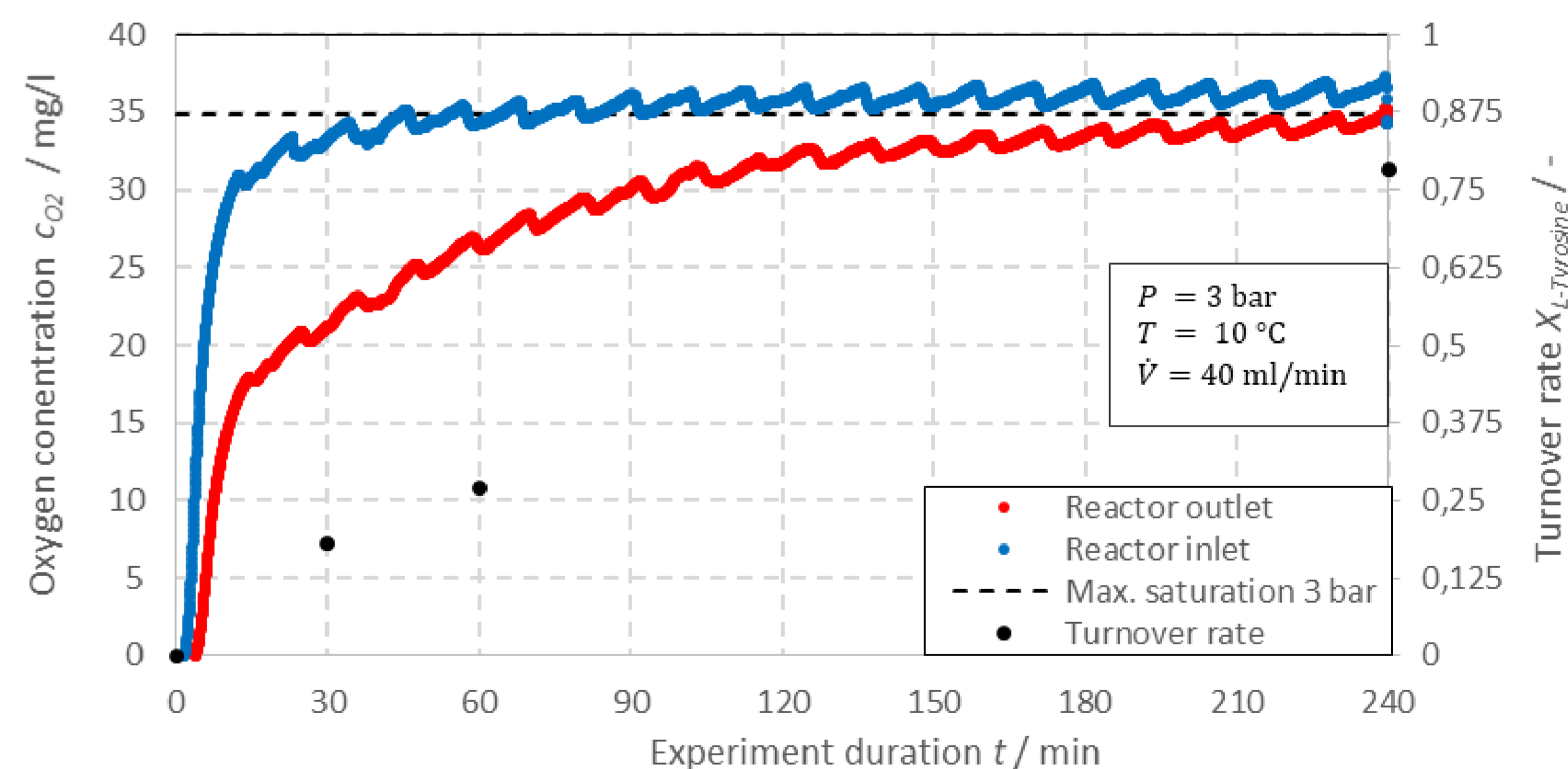


Fig. 4: Oxygen consumption by the enzyme compared to the supplied oxygen.

- For the immobilization of the enzymes:
  - Adapted 3D printed structures have been developed.
  - An immobilization method could be successfully established.

## Future Work & Outlook

- Deeper characterization of the prototype up to pressures of 150 bar.
- Scale up of the laboratory setup to pilot scale.
- Integration of the sensor technology into 3D printed carrier structure.

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### References:

- [1] Eisenmenger, M.J. et al.; Enzyme Microb. Technol. 2009, 45, 118-125.
- [2] Berheide, M. et al.; Biotechnol. Bioeng. 2010, 106, 18-26.
- [3] Ren, Y. et al.; BMC Biotechnol. 2011, 11, 63.
- [4] Spille, C. et al.; Chemrxiv 2020, Preprint. doi: 10.26434/chemrxiv.12152679.v1.